EFFECTS OF DIMETHYLSULFOXIDE ON PRODUCTIVITY OF X-IRRADIATED FLOUR BEETLES[1]

HOWARD E. ERDMAN

Biology Department, Pacific Northwest Laboratory, Battelle-Northwest, A Division of Battelle Memorial Institute, Richland, Washington

Chemical and physical agents are known to modify the degree of injury to biological systems subjected to radiation. Some of these agents by themselves might induce genetic damage (chromosomal aberrations) which may lead to illness or death of the organism. Successful eradication of the screw worm fly from Curaçao Island and the southeastern United States, by releasing males sterilized by gamma radiation (Baumhover *et al.,* 1955), has stimulated parallel research using chemical sterilants. The literature concerns predominately applied aspects for insect control (see reviews: Weidhaas and McDuffie, 1963; Smith, 1963; and Smith *et al.,* 1964).

A chemosterilant effect (inhibited ovarian growth) was reported for folic acid antagonists in *Drosophila* (Goldsmith and Frank, 1952), for various mitotic poisons and tumor-inhibiting agents in house flies (Mitlin and Baroody, 1958), and for alkylating agents in house flies (LaBrecque, 1961), screw worm flies (Crystal and LaChance, 1963), and mice (Moutschen, 1961). Detailed studies of the chemosterilant effect on insect oogenesis were found for fruit flies (Cantwell and Henneberry, 1963), house flies (Morgan and LaBrecque, 1962) and mosquitos (Rai, 1964). Effort is expanding to study the biological effects of chemosterilants and the mechanisms involved which result in modified insect productivity.

On the other hand, chemicals are sought for their radioprotective abilities. Ashwood-Smith (1961a) reported a 70% radioprotection of dimethylsulfoxide (DMSO) for whole-body acute x-ray exposures to mice.

The present paper reports that DMSO showed no radioprotective properties on oogenesis in flour beetles but had an indirect chemosterilant influence on flour beetle productivity. Microscopic examination of ovarioles showed that oogonial differentiation and growth were adversely affected, due to starvation resulting from unpalatability of food containing DMSO.

Materials and Methods

General

In these experiments, sexually-mature (three-week-old), virgin female flour beetles, *Tribolium castaneum* (Herbst), mutant: sooty, from the laboratory of Dr. A. Sokoloff, Berkeley, California, were treated with DMSO. After x-irradiation, females were mated to control males of the same age. X-irradiation was performed

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at 250 kvp, 30 ma, H.V.L. 0.86 mm. copper, 0.25 mm. copper + 1.0 mm. aluminum filtration and an anode-subject distance of 2.5 inches, and 1 kr/min. was delivered, as measured in air by a Victoreen Dosimeter. The flour beetles were maintained in conventional food (5% brewer's yeast, 95% whole-wheat flour) and at 32 ± 1° C. and 65–70% relative humidity. Dimethylsulfoxide, considered to have a density of 1, was diluted in distilled water.

Fecundity (mean number of eggs/♀/day), fertility (mean number of adult F_1/♀/day), and viability (total number of adult F_1/total number of eggs) were the productivity parameters measured. Dissections of ovarioles and whole-mount photomicrographs present the details of oogenesis.

Experiment 1

Sooty females were placed for 5 minutes on filter paper wetted with 0, 1, 10, and 100% DMSO, then x-rayed ½ hour after DMSO treatment with 0, 1, 2, and 4 kr. Wetted filter paper was used in order to increase the time of exposure and to eliminate drowning which occurred when beetles were dipped. Triplicate samples of five pairs in 10 g. of food were made and fresh food was given three times weekly during a 12-day period. At these times, eggs were counted and allowed to develop into adults, which were counted one month later.

Experiment 2

Sooty females were dipped three minutes in 67% DMSO. Three hours later they were exposed to 0, 2, and 4 kr. Subsequently, productivity measurements similar to those in Experiment 1 were made on five single pairs per treatment for 14 days.

Experiment 3

Sooty females were placed for 10 minutes on filter paper wetted with 67% DMSO. After 15 minutes and after 20 hours, they were x-irradiated with 0, 1, 2, and 4 kr. Three replicates of 10 pairs per 10 g. food were observed for 18 days under conditions similar to the other experiments, except that only the number of adult F_1 was counted.

Experiment 4

Sooty females were placed for four days on food containing 0, 4.6, and 9.1% DMSO, after which they were x-rayed with 0, 2, and 4 kr, and then mated. Five replicates of single pair-matings of these beetles were established on food containing no DMSO and thereafter lethality and productivity as described in Experiment 1 were studied.

Experiment 5

This experiment was repeated and the results were comparable; therefore, the data are treated as one experiment. Sooty females were kept for 0, 1, 4, and 11 days on food containing 0, 4.6, and 9.1% DMSO or no food. On these days,

females were preserved in 70% alcohol until dissection in insect Ringer solution. More than 20 whole-mount dissections were made and representative examples were photomicrographed.

Results

Productivity Data

Experiment 1

Fecundity, fertility, and viability progressively decreased with 2- and 4-kr x-ray exposures to approximately the same extent regardless of DMSO treatment (Table

Table I

PRODUCTIVITY MEASUREMENTS
Sexually Mature (3-week old) Virgin Female Flour Beetles, Tribolium castaneum (Herbst), mutant: sooty, X-Rayed One-Half Hour After 5 Minutes on Filter Paper Wetted with Dimethylsulfoxide (DMSO)

	X-Ray in kR			
% DMSO	0	1	2	4
	FECUNDITY (mean numbers of eggs/ ♀ /day)			
0	15 ± 0.4*	14 ± 0.7	13 ± 0.2	8 ± 0.5
1	13 ± 0.2	11 ± 0.4	11 ± 0.6	7 ± 0.5
10	14 ± 0.3	13 ± 1.4	11 ± 0.2	7 ± 0.5
100	All Died			
	FERTILITY (mean numbers of adult F_1/ ♀ /day)			
0	12 ± 0.4*	10 ± 0.9	6 ± 0.4	0.6 ± 0.005
1	11 ± 0.7	8 ± 0.8	4 ± 0.5	0.5 ± 0.1
10	12 ± 0.1	10 ± 1.0	5 ± 0.2	0.7 ± 0.1
	VIABILITY (fertility/fecundity)			
0	0.80 ± 0.03*	0.71 ± 0.05	0.46 ± 0.02	0.08 ± 0.01
1	0.85 ± 0.03	0.72 ± 0.03	0.36 ± 0.02	0.07 ± 0.03
10	0.86 ± 0.01	0.77 ± 0.01	0.46 ± 0.02	0.10 ± 0.03

*Standard Error

I). These parameters of productivity were not modified by DMSO for a given x-ray exposure. A 1-kr exposure resulted in responses comparable to control values.

X-radiation was highly significant in altering each productivity datum (Table II); DMSO was a statistically significant factor because of low productivity values

TABLE II

ANALYSIS OF VARIANCE
Square Root Transformation of Productivity Data from Sexually Mature (3-week old) Virgin Female Flour Beetles, Tribolium castaneum (Herbst), Mutant: Sooty, Placed for 5 Minutes on Filter Paper Wetted with Dimethylsulfoxide (DMSO) and X-Rayed One-Half Hour Later.

Error Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value
Fecundity				
1-DMSO	2	0.44	0.22	9.35 *
2-X-Ray	3	5.43	1.81	77.55 *
1-2 Interaction	6	0.07	0.01	0.49
Fertility				
1-DMSO	2	0.59	0.29	10.82 *
2-X-Ray	3	37.95	12.65	464.76 *
1-2 Interaction	6	0.17	0.03	1.07
Viability				
1-DMSO	2	0.05	0.03	6.96 *
2-X-Ray	3	4.52	1.50	398.17 *
1-2 Interaction	6	0.02	0.003	0.86

*Significant at the 5% Level

at 1% DMSO. Values at 0 and 10% DMSO were comparable; therefore, differences among values within a given x-ray exposure were considered as biological variability. No significant effects could be attributed to an interaction between x-ray and DMSO.

Experiment 2

A. Fecundity of females (Table III)

1. Not treated with DMSO: Comparable numbers of eggs were oviposited at 0- and 2-kr x-ray exposures.
2. Treated with DMSO: At 2- and 4-kr x-ray exposures, comparable numbers of eggs were oviposited which were significantly fewer compared to 0-kr values. Perhaps the dehydrant effect of DMSO impaired females in activity and/or oviposition.

B. Fertility (Table III)

Fertility of untreated and DMSO-treated females was progressively reduced to the same extent by increasing x-ray exposures but was not altered for a given x-ray exposure.

Table III

PRODUCTIVITY MEASUREMENTS
Sexually Mature (3-week old) Virgin Female Flour Beetles, *Tribolium castaneum* (Herbst), Mutant: Sooty, were X-Rayed 3 Hours After a 3 Minute Dip in 67 Percent Dimethylsulfoxide (DMSO)

	X-Ray in kR		
	0	2	4
% DMSO	FECUNDITY (mean number of eggs/ ♀ /day)		
0	17 ± 0.6*	18 ± 1.2	11 ± 2.3
67	21 ± 0.7	12 ± 1.5	11 ± 0.8
	FERTILITY (mean number of adult F_1/ ♀ /day)		
0	14 ± 0.9	9 ± 0.8	1 ± 0.1
67	15 ± 2.6	5 ± 1.6	1 ± 0.2
	VIABILITY (fertility/fecundity)		
0	0.82 ± 0.02	0.50 ± 0.03	0.09 ± 0.04
67	0.71 ± 0.1	0.41 ± 0.1	0.09 ± 0.03

*Standard Error

C. Viability (Table III)

For untreated or DMSO-treated females the numbers of eggs which developed into adults progressively decreased with greater x-ray exposures.

D. Analysis of Variance (Table IV)

1. X-irradiation and not DMSO was the effective agent in modifying the productivity responses of female flour beetles.
2. The F-value, 6.04, for the interaction between DMSO and x-rays was significant only for fecundity. This might be explained by the low mean number of eggs of DMSO-treated females given 2 kr.

TABLE IV

ANALYSIS OF VARIANCE
Square Root Transformation of Productivity Data from Sexually Mature (3-week old) Virgin Female Flour Beetles, Tribolium castaneum (Herbst), Mutant: Sooty, X-Rayed 3 Hours After a 3 Minute Dip in 67 Percent Dimethylsulfoxide (DMSO)

Error Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value
Fecundity				
1-DMSO	1	0.03	0.03	0.16
2-X-Ray	2	6.51	3.26	16.39*
1-2 Interaction	2	2.40	1.20	6.04*
Fertility				
1-DMSO	1	0.67	0.70	1.75
2-X-Ray	2	36.13	18.07	47.17*
1-2 Interaction	2	1.33	0.67	1.74
Viability				
1-DMSO	1	0.08	0.08	2.33
2-X-Ray	2	2.94	1.47	40.93*
1-2 Interaction	2	0.03	0.02	0.48

*Significant at the 5% Level

TABLE V

MEAN NUMBERS OF F_1 ADULTS PER FEMALE PER DAY WHEN SEXUALLY MATURE (3-WEEK OLD) VIRGIN FEMALE FLOUR BEETLES, Tribolium castaneum (HERBST), MUTANT: SOOTY, WERE PLACED FOR 10 MINUTES ON FILTER PAPER WETTED WITH DIMETHYLSULFOXIDE (DMSO) AND THEN X-RAYED 15 MINUTES OR 20 HOURS LATER.

	X-Ray in Kr			
% DMSO	0	1	2	4
X-Rayed After 15 Minutes				
0	14 ± 1.3*	11 ± 0.9	7 ± 0.5	1 ± 0.3
67	12 ± 1.1	11 ± 1.2	6 ± 0.7	1 ± 0.2
X-Rayed After 20 Hours				
0	13 ± 1.4	11 ± 0.9	7 ± 0.6	1 ± 0.2
67	11 ± 0.8	11 ± 0.9	7 ± 0.6	1 ± 0.3

* Standard Error

At 4 kr the productivity measurements for untreated and DMSO-treated females were significantly and comparably reduced compared to those of other radiation exposures, indicating radiation induced irreparable damage to developing mature oocytes. Likewise, the 2-kr exposure reduced fertility and viability significantly from control values.

Experiment 3

A. Fertility (Table V)
   1. Was the same for 0 and 1 kr but was progressively reduced by 2 and 4 kr.
   2. Was comparable for untreated and DMSO-treated females given a specified x-ray exposure.
   3. Was not changed whether x-ray was given 15 minutes or 20 hours after DMSO treatment.

Experiment 4

In addition to results previously stated concerning x-ray effects on productivity, data of this experiment (Table VI) showed that flour beetle females subjected

Table VI

PRODUCTIVITY DATA SUMMARY FOR SEXUALLY MATURE (3-WEEK OLD) VIRGIN FEMALE FLOUR BEETLES, *Tribolium castaneum* (HERBST), MUTANT: SOOTY, FED FOUR DAYS ON FOOD CONTAINING DIMETHYLSULFOXIDE (DMSO)

% DMSO	Kr X-Ray	1	2	3	2	5	3	Percent Parental	
		Number of Days in Productivity Period						Lethality	Fertility
0	0	5/5 *	5/5	5/5	5/5	5/5	5/5	0	100
		5 **	15	16	17	18	17		
4.6	0	0/5	5/5	5/5	5/5	5/5	5/5	0	100
		0	8	13	15	12	13		
9.1	0	0/5	0/5	1/3	2/3	2/3	2/3	40	40
		0	0	21	17	15	18		
0	2	5/5	5/5	5/5	5/5	5/5	5/5	0	100
		5	8	5	6	6	7		
4.6	2	0/5	3/5	3/5	3/5	3/5	3/5	0	60
		0	2	8	10	6	6		
9.1	2	0/5	0/5	1/2	1/2	1/2	1/2	60	20
		0	0	5	4	5	4		
0	4	3/5	5/5	5/5	2/5	2/5	0/5	0	100
		1	3	0.7	0.2	0.1	0		
4.6	4	0/5	1/5	2/4	0/4	2/4	0/4	20	40
		0	2	2	0	0.4	0		
9.1	4	0/5	0/5	0/3	0/3	0/3	0/3	40	0
		0	0	0	0	0	0		

* Number Reproducing ♀♀/Number ♀♀Tested

** $\bar{X}$ Number of Adult F_1/ ♀ /day.

to food containing DMSO for four days and then x-rayed had: (1) increased lethality at 9.1% DMSO; (2) progressively decreased fertility; (3) delayed onset of productivity by one day at 4.6% DMSO and at least three days at 9.1% DMSO. Delay of reproductive onset was found also when no x-ray treatment was given; therefore, this response was not due to x-radiation but to DMSO-contaminated food resulting in failure to feed and oosorption. These females were not observed cytologically since subsequent production of F_1 progeny was indicative of functioning ovarioles. Females on 9.1% DMSO then given 4 kr never reproduced because the radio-resistant mature oocytes were resorbed, due to the unpalatibility of DMSO on food, and the radiosensitive immature oocytes were irreparably damaged by this quantity of x-ray.

Ovariole Cytology

Experiment 5

Representative ovarioles of flour beetles sacrificed after 0, 1, 4, and 11 days on control food (no DMSO) are shown in Figure 1. In all dissections, a female had paired ovaries, each one of which consisted of 6 ovarioles. Progressing distally, one finds large (mature) oocytes ready for oviposition, oocytes in different stages of growth, differentiating oocytes, the gametorium, and the terminal filament. Similar ovarioles were observed in females cultured for one day on food containing 4.6% or 9.1% DMSO or kept without food. Thus, regardless of treatment during this time, no differences appeared in the size or number of ovarioles or oocytes.

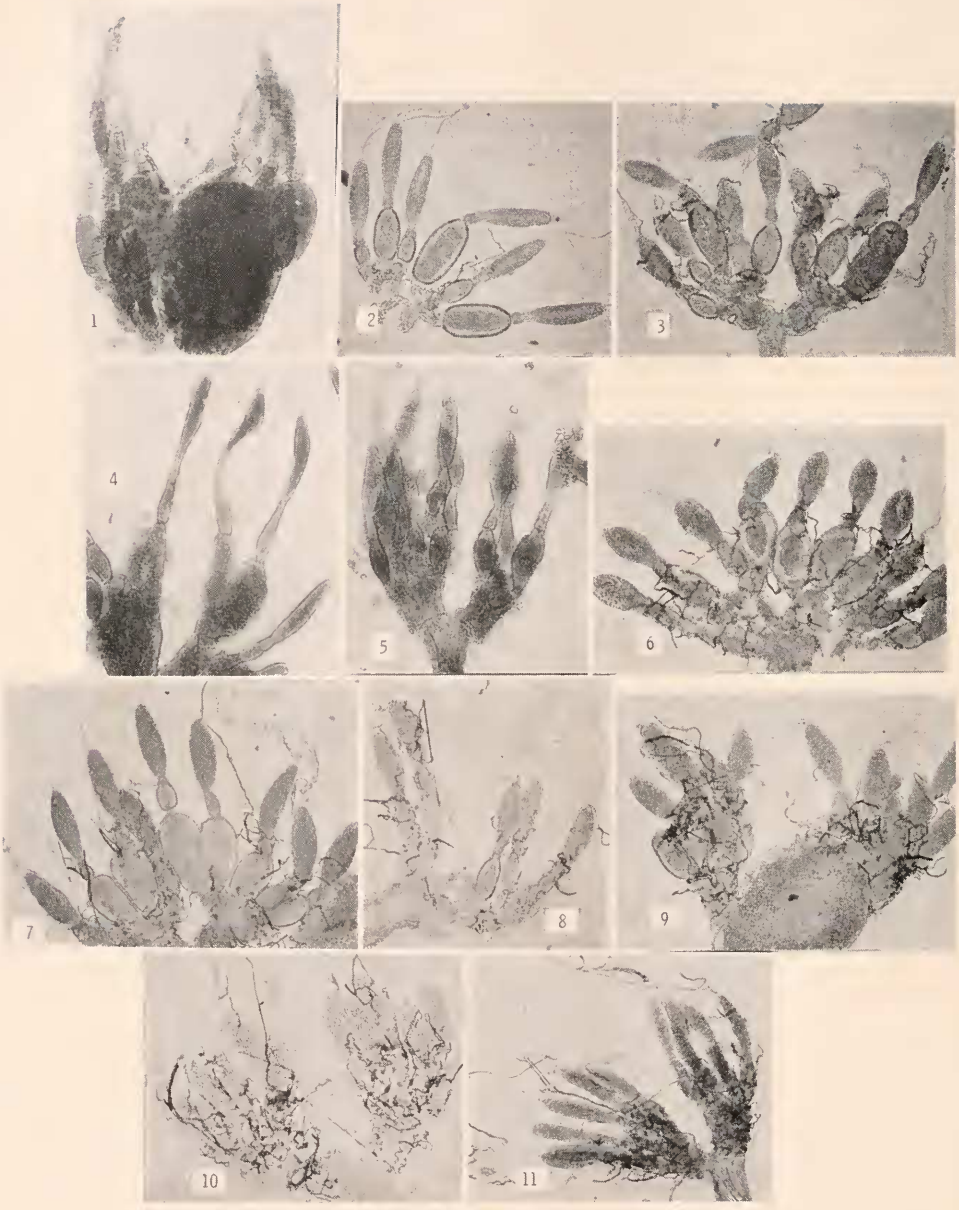
As expected from the productivity data in Experiment 4, ovariole deterioration was observed in females which spent four days on food containing 4.6% DMSO (Figs. 4, 5). In a few females, large oocytes were observed which could, perhaps, have been oviposited. When oocytes no longer differentiated (after four days), the ovarioles appeared thin, shortened, and empty (Figs. 8, 9, 11). Comparable observations were found in females kept four days on 9.1% DMSO in food (not shown). No oocyte appeared mature enough for oviposition and ovariole degeneration was pronounced. Since some (two out of five) females produced a few F_1 (total of 24 per reproducing female during 15 days), some germ cells survived (Table VI).

Compression of the beetle abdomens indicated starvation, which resulted in sterility due to oosorption. Ovarioles from beetles kept without food and dissected on days 3, 5, and 11 (Figs. 2, 3, 6, 7, 10) showed no differences from those of beetles kept on food containing DMSO (Figs. 4, 5, 8, 9, 11). Thus females of *Tribolium* kept without food or unpalatable food absorb mature eggs and fail to mature others.

Discussion

Topical application of DMSO gave no protection to female flour beetle germ cells subjected to x-irradiation. The high (70%) radiation-protective ability of DMSO in mice exposed to whole-body acute lethal x-radiation (Ashwood-Smith, 1961a) was not found to decrease x-ray damage in mice testes, even though it was considerably concentrated in the testes (Ashwood-Smith, 1961b). An attempt to subject female flour beetles to low concentrations, 4.6% and 9.1%, of DMSO over a long period of time (days *vs.* minutes) showed that productivity was reduced after four days. Three out of five females given 9.1% DMSO in their food but not x-rayed never regained their reproductive ability. Of the two productive females, one began reproduction during days 4–6 and the other during days 7–8. Two of the three females not producing died during the first three days. Decreased fecundity might result from chemosterilant-induced interference with the progress of normal oogenesis.

Some biological alkylating agents, such as tepa (Borkovec, 1962), are the most effective male insect chemosterilants—eggs laid by females mated to treated males failed to hatch. These agents were used to sterilize both sexes of species of mosquitos, *Aedes* (Weidhaas *et al.*, 1961; Bertram, 1963; Rai, 1964) and *Culex* (Murray, 1963), females of house flies (LaBrecque, 1961; Morgan and LaBrecque, 1962), of screw worm flies (Chamberlain, 1962) and of *Drosophila* (Cantwell and Henneberry, 1963). Widespread use of these chemicals is restricted because of



FIGURES 1–11.

Whole-mount photomicrographs (82 × magnification) of representative ovarioles of sexually mature flour beetles, *Tribolium castaneum* (Herbst), mutant: Sooty, cultured at 32° C. and 65–70% relative humidity. Figure 1, from control flour beetles on days 1 and 11; Figures 2 and 3, from flour beetles kept three days without food; Figures 4 and 5, from flour beetles kept four days on food containing 4.6% DMSO; Figures 6 and 7, from flour beetles kept without food for 5 days; Figures 8 and 9, from flour beetles kept 8 days on food containing 4.6% DMSO; Figure 10, from flour beetles kept 11 days without food; Figure 11, from flour beetles kept 11 days on food containing 4.6% DMSO.

their possible mutagenic and carcinogenic properties (Auerbach, 1958). Chang *et al.* (1964) reported effective male house fly chemosterilants, hexamethylphosphoramide and hexamethylmelamine, which are non-alkylating agents and have low mammalian toxicity. Compared to these chemicals, the unrelated and simple molecular structure of dimethylsulfoxide—$H_3C—\overset{\uparrow}{\underset{O}{S}}—CH_3$—was not expected to affect gametogenesis adversely.

Critical examination of the females given food containing DMSO revealed the contracted appearance of their abdomens and suggested that starvation resulting in oosorption caused the decreased productivity, rather than DMSO *per se*. Observations of ovarioles from females kept without food were the same as those from females subjected to DMSO-food. Ovarioles from both treatments progressively deteriorated with time.

Microscopic examinations indicated that flour beetle ovarioles were panoistic; however, to be conclusive this will be studied in more detail by incorporating cytochemical techniques. Panoistic ovarioles are distinguished from meroistic ovarioles in that the former have no trophocytes. Types of meroistic ovarioles are: (1) telotrophic, having trophocytes in the germarium; and (2) polytrophic, having syncytia each consisting of trophocytes and an egg cell enclosed in cellular follicles. In the apparent absence of nutritive cords (Bryan, 1954), a detailed study of oogenesis is required to determine whether the apical cells in the germarium are oocytes or trophocytes. Stein (1847), Gross (1903), and Imms (1948) reported polytrophic ovarioles for the coleopteran suborder *Adephaga* and telotrophic ovarioles for the rest of the beetles. On the other hand, Weber (1933) and Wigglesworth (1950) reported telotrophic ovarioles for the *Adephaga* and polytrophic ovarioles for the *Polyphaga*. Bonhag (1958) concluded the existence of panoistic ovarioles in the Coleoptera was not established.

Concentrations as low as 4.6% DMSO indirectly caused sterility by making the food unpalatable and by the subsequent utilization of oocytes as an energy source. Preliminary observations of approximately 100 adult beetles from stock cultures to determine the concentration of DMSO allowing propagation after 5 weeks showed that no F_1 were obtained on 2.3% DMSO food, three F_1 were obtained on 1.8% and 1.4% DMSO food and more than 100 F_1 were produced on 0.9% DMSO food. The 2.3% DMSO food induced 95% lethality in adults after three weeks. Due to these findings, future work is planned to test the usefulness of DMSO as an insecticide or insect repellant on stored products such as foods, clothes, and tobacco.

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Summary

Sexually mature virgin female flour beetles, *Tribolium castaneum* (Herbst), mutant: sooty, were treated with dimethylsulfoxide (DMSO) and x-rayed. Fecun-

dity, fertility and viability were measured. DMSO gave no radiation protection to female germ cells. Food containing 4.6% DMSO was unpalatable. Photomicrographs of ovarioles showed that sterility resulted because of oosorption. DMSO might be economically important in protecting stored products from insects.

LITERATURE CITED

Ashwood-Smith, M. J., 1961a. The radioprotective action of dimethyl sulphoxide and various other sulphoxides. *Int. J. Radiation Biol.,* **3**: 41–48.

Ashwood-Smith, M. J., 1961b. Inability of dimethyl sulphoxide to protect mouse testis against the effects of X-radiation. *Int. J. Radiation Biol.,* **3**: 101–103.

Auerbach, C., 1958. Mutagenic effects of alkylating agents. *Ann. N. Y. Acad. Sci.,* **68**: 731–736.

Baumhover, A. H., A. J. Graham, B. A. Bitter, D. E. Hopkins, W. D. New, F. H. Dudley and R. C. Bushland, 1955. Screw-worm control through release of sterilized flies. *J. Econ. Entomol.,* **48**: 462–466.

Bertram, D. S., 1963. Observations on the chemosterilant effect of an alkylating agent, thio-tepa, on wild-caught *Anopheles gambiae* var. *melas* (Theo.) in Gambia, West Africa and on laboratory bred *A. g. gambiae* giles and *Aedes aegypti* (L.) *Trans. Roy. Soc. Trop. Med. Hyg.,* **57**: 322–335.

Bonhag, P. F., 1958. Ovarian structure and vitellogenesis in insects. *Ann. Rev. Entomol.,* **3**: 137–160.

Borkovec, A. B., 1962. Sexual sterilization of insects by chemicals. *Science,* **137**: 1034–1037.

Bryan, J. H. D., 1954. Cytological and cytochemical studies of oogenesis of *Popilius disjunctus* Illiger (Coleoptera-Polyphaga). *Biol. Bull.,* **107**: 64–79.

Cantwell, G. E., and T. J. Henneberry, 1963. The effects of gamma radiation and apholate on the reproductive tissues of *Drosophila melanogaster* Meigen. *J. Insect. Pathol.,* **5**: 251–264.

Chamberlain, W. F., 1962. Chemical sterilization of the screw-worm. *J. Econ. Entomol.,* **55**: 240–248.

Chang, S. C., P. H. Terry and A. B. Borkovec, 1964. Insect chemosterilants with low toxicity for mammals. *Science,* **144**: 57–58.

Crystal, M. M., and L. E. LaChance, 1963. The modification of reproduction in insects treated with alkylating agents. I. Inhibition of ovarian growth and egg production and hatchability. *Biol. Bull.,* **125**: 270–279.

Goldsmith, E. D., and I. Frank, 1952. Sterility in the female fruit fly, *Drosophila melanogaster,* produced by the feeding of a folic acid antagonist. *Amer. J. Physiol.,* **171**: 726–727.

Gross, J., 1903. Untersuchungen über die Histologie des Insectenovariums. *Zool. Jahrb. Abt. Anat. u. Ont.,* **18**: 71–186.

Imms, A. D., 1948. A General Textbook of Entomology. E. P. Dutton and Co., New York, N. Y.

LaBrecque, G. C., 1961. Studies with three alkylating agents as house fly sterilants. *J. Econ. Entomol.,* **54**: 684–689.

Mitlin, N., and A. M. Baroody, 1958. Use of the housefly as a screening agent for tumor-inhibiting agents. *Cancer Res.,* **18**: 708–710.

Morgan, P. B., and G. C. LaBrecque, 1962. The effect of apholate on the ovarian development of houseflies. *J. Econ. Entomol.,* **55**: 626–628.

Moutschen, J., 1961. Differential sensitivity of mouse spermatogenesis to alkylating agents. *Genetics,* **46**: 291–299.

Murray, W. S., 1963. The effect of apholate on the mosquito *Culex pipiens quinquefasciatus* Say. *Entomol. Soc. Amer. Bull.,* **9**: 173.

Rai, K. S., 1964. Cytogenetic effects of chemosterilants in mosquitos. II. Mechanism of apholate-induced changes in fecundity and fertility of *Aedes aegypti* (L.). *Biol. Bull.,* 127: 119–131.

Smith, C. N., 1963. Prospects for vector control through sterilization procedures (in Vector Control). *Bull. World Health Organization Supp.*, **29**: 99–106.

Smith, C. N., G. C. LaBrecque and A. B. Borkovec, 1964. Insect chemosterilants. *Ann. Rev. Entomol.*, **9**: 269–284.

Stein, F., 1847. Vergleichende Anatomie und Physiologie der Insecten, I. Die weiblichen Geschlechtsorgane der Käfer. Duncker u. Humblot, Berlin, 139 pp.

Weber, H., 1933. Lehrbuch der Entomologie. Fischer, Jena.

Weidhaas, D. E., H. R. Ford, J. B. Graham and C. N. Smith, 1961. Preliminary observations on chemosterilization of mosquitoes. *N. J. Mosquito Extermin. Assoc. Proc.*, **48**: 106–109.

Weidhaas, D. E., and W. C. McDuffie, 1963. Highlights of recent research on chemosterilants for the control of insects of medical and veterinary importance. *Entomol. Soc. Amer. Bull.*, **9**: 268–272.

Wigglesworth, V. B., 1950. The Principles of Insect Physiology. Methuen and Co., Ltd., London, England.